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Full Length Research Paper

***In vitro* investigation of the hypoglycemic activity of yeasts using models of rat epididymal adipocyte and differentiated mouse 3T3-L1 adipocyte**

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The differentiated mouse 3T3-L1 adipocytes (3T3-L1 model) were used in studying glucose metabolisms without the need for feeding (Sprague-Dawley, SD model) the rat prior to hypoglycemic activity evaluation. Both models were adopted to evaluate the hypoglycemic activities of 58 yeast strains isolated from various sources (grape, vine yard soil, winery soil). Among the 58 tested yeast isolates, strain 54 (*Saccharomyces pastorianus* no. 54) which showed the highest hypoglycemic activity was chosen to be the test strain. The optimal insulin concentration used in these 2 models (SD and 3T3-L1) for measuring the hypoglycemic activity of hypoglycemic yeast extract (HGYE) was 10 nM. The range of linear relation in the dose-response curve was 0-1000 g/ml for SD model, and 0-250 g/ml for 3T3-L1 model. The linear coefficient was 0.8611. The radioactive labeled 2-[1-¹⁴C]-Deoxy-D-Glucose was also used to confirm cytoplasmic glucose uptake by 3T3-L1 adipocytes. Comparing both the results of insulin effect and dose response of HGYE by both models, it was concluded that the 3T3-L1 model can serve as a rapid and reliable assay model for *in vitro* evaluation of hypoglycemic activity of yeast.

Key words: 3T3-L1 adipocytes, Sprague-Dawley (SD) rat, epididymal adipocytes, hypoglycemic activity, yeast.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders in which a person has high blood sugar because the body either lacks insulin or is resistant to the hormone (Atkinson and Maclaren, 1994; Tisch and McDevitt, 1996; Jeffcoate, 2004). People with DM are considered to be at high risks of atherosclerotic cardiovascular disease (Atkinson and Maclaren, 1994; Tisch and McDevitt, 1996),

renal failure, blindness, and limb amputation (Jeffcoate, 2004). Data from the global DM prevalence analysis for the year 2000 demonstrated that more than 170 million people worldwide are affected by this disease, and this number is predicted to be over 365 million people by the year 2030 (Wild et al., 2004). Searching for appropriate hypoglycemic substances has been intensively investigated by many researchers. However, reports regarding the hypoglycemic extracts from microorganisms (Davies et al., 1985; Yadav et al., 2007) are fairly limited, even though their production cost is much lower than those derived from plant materials. It has been reported that the hypoglycemic extracts can be isolated from brewer's yeast (Davies et al., 1985), probiotics (Yadav et al., 2007), *Monascus* (Su et al., 2007) and *Ganoderma* (Jia et al., 2009).

Chromium containing hypoglycemic extracts from Brewer's yeast was first identified by Schwarz's research

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Abbreviations: HGYE, Hypoglycemic yeast extract; SD, Sprague-Dawley; DX, dexamethasone; IBMX, 3-isobutyl-1-methylxanthine; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; 2-DG, 2-(1-¹⁴C)deoxy-D-glucose.

group (Mertz and Schwarz, 1955). They discovered that the rats fed with the *Torula* yeast diet supplemented with the extract of Brewer's yeast had lower level of blood glucose, indicating this extract could help rats to maintain normal blood glucose and prevented the rats from glucose metabolism impairment (Mertz and Schwarz, 1955; Schwarz and Mertz, 1959; Mertz et al., 1961). The functional component in such extract was called the glucose tolerance factor (GTF) since then. Simonoff et al. (1992) showed that the supernatant of homogenized Brewer's yeast could stimulate *in vitro* glucose oxidation of the epididymal adipocyte of Sprague-Dawley (SD) rat in the presence of insulin. Holdsworth and Appleby (1984) used radioactive ^{14}C to prove that yeast extract could enhance the reactions of the decarboxylation and production of CO_2 and alcohol in yeast.

The aforementioned studies exploited the Sprague-Dawley rat (SD model) for *in vitro* evaluation of hypoglycemic activity. However, using an animal model is normally time-consuming, and is restricted to limited animal sources, while sacrificing the lives of animals. Therefore, the differentiated 3T3-L1 adipocyte (3T3-L1 model), which could serve the same purpose as the SD model in glucose uptake, was developed. Liu et al. (2001) discovered that hot water extraction of *Lagerstroemia speciosa* L. (banaba) from Philippine possessed hypoglycemic activity in the *in vitro* stimulation of the glucose uptake of differentiated 3T3-L1 cells (Liu et al., 2001). Similarly, Yaworsky et al. (2000) found that α -lipoic acid could stimulate the glucose intake of 3T3-L1 adipocytes. The 3T3-L1 model has the advantage of avoiding the use of radioactive glucose.

In this study, the preadipocyte 3T3-L1 was differentiated with Dexamethasone (DX), insulin and 3-isobutyl-1-methylxanthine (IBMX) and was used for screening yeast extracts with hypoglycemic activity. The 3T3-L1 model was compared with the SD model, and the results of the two models were shown to be highly correlated in terms of hypoglycemic activity. The effects of insulin concentration and dose response of yeast extract between these two models for measuring the hypoglycemic activity of yeast extract were also compared.

MATERIALS AND METHODS

Male Sprague-Dawley (SD) rats (3 week-old, body weight approximately 80 g) were purchased from the National Laboratory Animal Center (NLAC, Taipei, Taiwan). The 3T3-L1 preadipocytes (BCRC CL-173) were obtained from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan). Insulin, dexamethasone, and 3-iso-butyl-1-methylxanthine were obtained from Sigma (St. Louis, MO, USA). Malt extract broth (MEB), malt extract agar (MEA) and peptone were purchased from Becton Dickinson Company (Sparks, MD, USA). Dulbecco's modified Eagle's medium (DMEM) was obtained from Invitrogen Corporation (Carlsbad, CA, USA). Fetal bovine serum (FBS) was purchased from PAA Laboratories (Linz, Austria). Wako L-type TG H test was purchased from Wako Corporation (Tokyo, Japan). The radioactive 2-[1- ^{14}C]-Deoxy-D-Glucose was purchased from Perkin-Elmer Life and

Analytical Science (Boston, MA, USA).

Collection of yeast isolates

Samples of grapes from local market place, soils from vineyard and wineries in Nantou County in Taiwan, were collected and stored in sterile bags. After arrival at laboratory, samples were homogenized and diluted with 0.1% peptone water, followed by streak plating on malt extract agar (MEA) containing 50 ppm chlortetracycline and 50 ppm chloramphenicol. Colonies grown on MEA plate were purified by streaking method and then preserved on MEA slant at 4°C.

Culture condition

Based on the methods of Tsai and Liu (1983) and Tsai et al. (1984) with minor modification, collected yeast isolates were subcultured twice in MEB medium at 25°C for 2 days. 1 ml of each culture was then inoculated to 300 ml MEB and incubated at 25°C with shaking at 150 rpm for 4 days. After centrifugation (5,000×g, 20 min) and washing twice with de-ionized water, the yeast precipitate was collected and then freeze-dried.

Preparation of yeast extract

Based on the method of Mertz et al. (1995), one gram of freeze-dried yeast powder was added to 20 ml of 0.1 N NH_4OH aqueous solutions and incubated at 30°C in water bath with shaking for 2 h. After centrifugation (10,000×g, 15 min), the supernatant was collected and freeze-dried. The freeze-dried hypoglycemic yeast extract (HGYE) was subsequently used for hypoglycemic activity evaluations.

Hypoglycemic activity analysis by SD model

Based on the method of Davies et al. (1985), male Sprague-Dawley (SD) rats (3 week-old, purchased from National Laboratory Animal Center, ROC) were fed with normal diet for 1 week, and then switched to *Torula* yeast diet (TestDiet, Richmond, USA) for 4 weeks. Fat cells from rats were isolated by the method of Rodbell (1964) with some modifications. Rats were sacrificed by decapitation, the epididymal fat pads were removed and rinsed with 0.85% NaCl solution, and thin distal portions from each pad were divided into three parts. One gram of fat pad tissue was added to a siliconized 25 ml flask containing 3 ml of albumin-bicarbonate buffer (pH 7.4), 10 mg/ml of collagenase, and 3 $\mu\text{moles/ml}$ of glucose, and was incubated at 37°C for 1 h. The cells were filtered by homogenizer collector (300 mesh / 46 μm) and centrifuged (400×g, 1 min). The sediment cells were washed three times by KRB buffer (118 mM NaCl, 4.7 mM KCl, 1.25 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2 mM KH_2PO_4 , 25 mM NaHCO_3 and 15 mM HEPES, pH 7.2-7.4).

Cell suspension (10⁵ cell/ml) was inoculated in the 6-well plate, each well contains 200 μl HGYE (1 mg/ml) and 200 μl KRB buffer with 10 nM insulin and glucose 2.5 g/L, and was incubated in 5% CO_2 atmosphere at 37°C for 2 h. After the 2 h incubation, 1 ml culture medium was taken for centrifugation (400×g, 10 min) and the supernatant was then collected for glucose determination using a glucose analyzer (YSI-2700, YSI Incorporated, Yellow Spring, USA). The glucose uptake of cells was determined by the difference between the initial and the final glucose concentrations. The relative increased glucose uptake percentage was calculated as follows;

Increased glucose uptake percentage (%) = [(glucose uptake of cells with HGYE - glucose uptake of control) / glucose uptake of control] × 100%

Cell culture

3T3-L1 preadipocyte (BCRC CL-173), obtained from the Bioresource Collection and Research Center, Hsinchu, Taiwan, was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin (10,000 U/ml penicillin and 10,000 µg/ml streptomycin in 0.85% saline). Culture conditions were 37°C and an atmosphere of 5% CO₂. The culture medium was refreshed every two days.

Cell culture differentiation

Based on the protocol of Student et al. (1980), the differentiation of 3T3-L1 preadipocytes was initiated with 1 µg/ml insulin, 1 µM dexamethasone and 0.5 mM 3-iso-butyl-1-methylxanthine in DMEM supplemented with 10% FBS. After incubation at 37°C for 48 h, the culture medium was replaced with DMEM supplemented with 10% FBS and 1 µg/mL insulin, and was incubated for an additional 48 h. Cells were then fed with DMEM containing 10% FBS every other day. The cells are fully differentiated into adipocytes at the end of day 10, as monitored by Oil Red-O staining (Jensen et al., 2004). The differentiated adipocytes were used for glucose uptake assay.

Oil Red-O staining

The staining method was based on Jensen et al. (2004). Cells were rinsed three times in phosphate buffered saline (PBS) and then fixed in 10% (v/v) paraformaldehyde for 1 min. Fixed cells were rinsed with deionized water, then stained with Oil Red-O for 30 min at room temperature, then rinsed again for three times with deionized water. The cells were counterstained with Harris's hematoxylin for 30 s for nuclear staining of adipocyte, followed by rinsing with deionized water. Images were photographed using an Olympus inverted microscope with a Canon 450D digital camera.

Cell triglyceride assay

Based on the method of Jensen et al. (2004), the differentiated adipocyte was harvested and rinsed three times with PBS; these cells were then scraped off the plates and transferred to 2 ml microcentrifuge tubes. Cells were added with a lysing buffer (0.25 M sucrose, 1 mM sodium-EDTA, 5 mM Tris, and 1 mM dithiothreitol, pH 7.4). The suspension was sonicated for 30 s, and then centrifuged (10000 xg, 20 min). The triglyceride content in the supernatant was measured using the Wako L-type TG H test (Wako, Tokyo, Japan).

Hypoglycemic activity analysis by 3T3-L1 cell line

The differentiated 3T3-L1 adipocytes in the microtiter plate were put to a state of fasting in DMEM without glucose at 37°C for 1 h in 95% air-5% CO₂. After being washed with PBS, 200 µl of HGYE (250 g/ml) and 200 µl KRB buffer containing 10 nM insulin and 2.5 g/L glucose were added. The cultures were further incubated at 37°C in 95% air-5% CO₂ for 2 h. After centrifugation (400xg, 15 min), glucose content in the supernatant, the glucose uptake of cells were obtained in the same way as those for the hypoglycemic activity analysis of SD model described in the aforementioned section.

The effect of insulin and HGYE concentrations

This part of the experiment was conducted in two stages. In stage 1, various concentrations of insulin (0, 5, 10, 25, 50 and 100 nM) were

added to the medium and incubated with rat epididymal adipocytes or 3T3-L1 adipocytes to obtain optimal insulin level. In stage 2, the insulin level was fixed at optimum and various HGYE (0, 125, 250, 500, 1000 µg/ml) were added to the medium. Their effects on increased glucose uptake percentage in both SD and 3T3-L1 models were determined as described earlier.

3T3-L1 cell analysis with ¹⁴C-labeled glucose

Based on method of Huang et al. (2009), the differentiated 3T3-L1 adipocytes were cultured in DMEM without glucose at 37°C for 1 h in 95% air-5% CO₂. After washing with PBS, 200 µl of HGYE (0, 125, 250, 500, 1000 µg/ml) and 200 µl KRB buffer containing insulin (0, 5, 10, 25, 50, 100 nM) were added and incubated at 37°C in 5% CO₂ atmosphere for another 2 h. 20 µl of 3T3-L1 cell suspension was amended with 40 µl DMEM without glucose and 20 µl 2-[1-¹⁴C]-Deoxy-D-Glucose (2-DG, 2.5 µCi/ml) and then incubated at 37°C for 10 min. The reaction was terminated on ice and then centrifugated (8,000xg, 3 min). The cell precipitate was collected and washed twice with PBS. 50 µl 1N NaOH was added and stood at 37°C for 1.5 h. Then, 50 µl 1N HCl was added to neutralize NaOH. The mixture was then transferred to the vial of a scintillation instrument (ASC; Amersham, Arlington Heights, IL), and mixed thoroughly with 3 ml scintillation fluid. The radioactivity was read by β-counter (Model LS5000CE, Beckman, CA). Specific 2-DG uptake was expressed as counts per minute per milligram of protein. Increased 2-DG uptake was calculated as follow:

$$\text{Increased 2-DG uptake (cpm/mg protein) (\%)} = \left[\frac{\text{specific 2-DG uptake with HGYE} - \text{specific 2-DG uptake of control}}{\text{specific 2-DG uptake of control}} \right] \times 100\%$$

Statistical analysis

All experiments were performed in triplicates. The results were expressed as mean ± SD (n=3). Statistical comparisons were made using the Duncan's multiple range tests. Differences were considered significant when P- values were below 0.05 (p < 0.05).

RESULTS AND DISCUSSION

In quantifying hypoglycemic activity of chromium (III) containing agents, the SD model has been widely adopted for years. In brief, the model involved feeding rats following specific schemes for 5 weeks, then, the epididymal fat pads of the rats were taken for *in vitro* analyses in which ¹⁴C labeled glucose was used to trace glucose uptake. The 3T3-L1 model proposed in this study involves only differentiation of the 3T3-L1 preadipocyte cells followed by *in vitro* glucose uptake measurement. The latter has bypassed the time-consuming feeding period, sacrificing expensive experimental rats, as well as using radioactive compounds. If validated, the 3T3-L1 model would be more desirable in glucose metabolism related studies.

Cell differentiation in 3T3-L1 model

The differentiation status of 3T3-L1 preadipocytes was

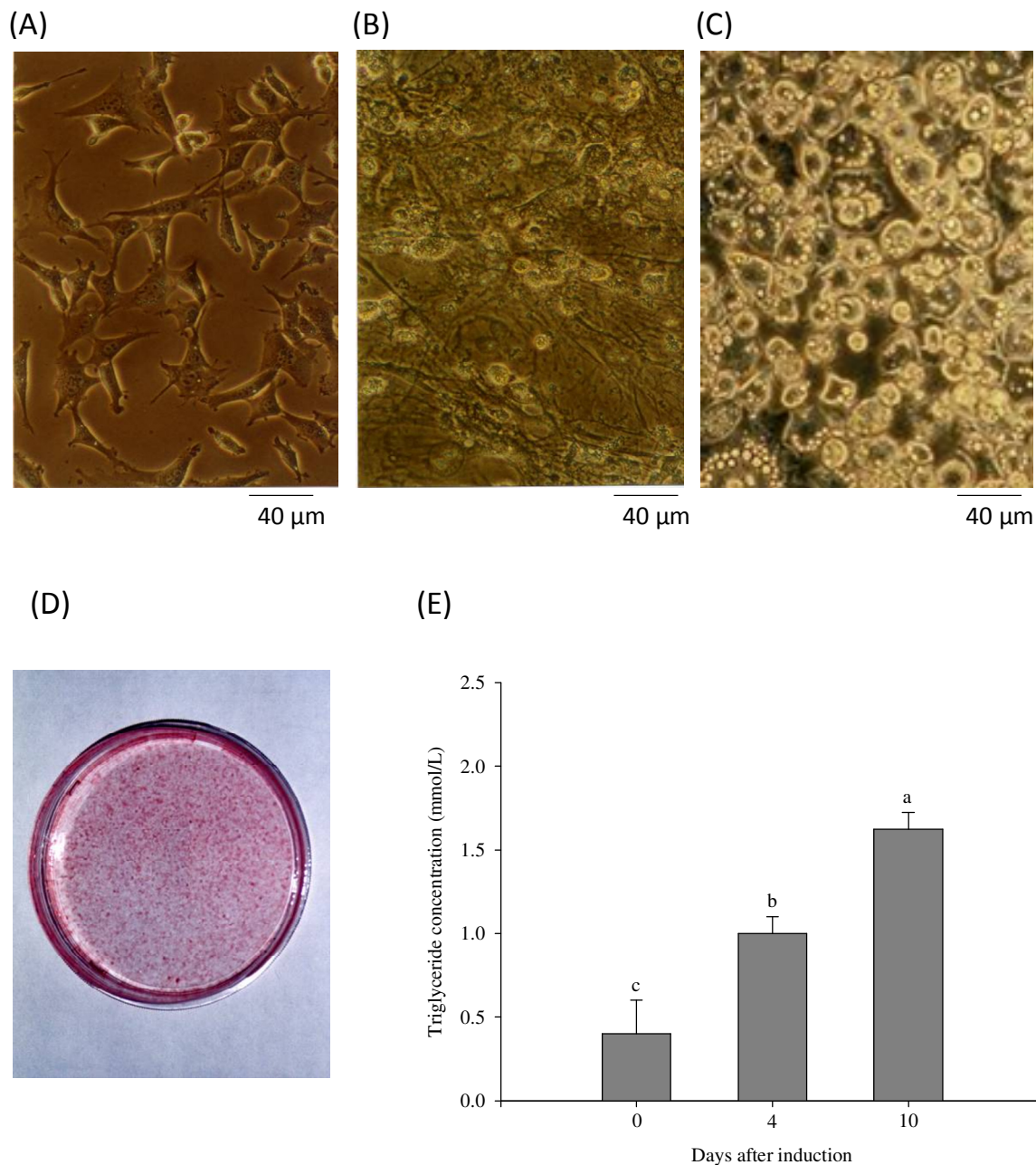


Figure 1. The differentiation of 3T3-L1 preadipocytes into adipocytes. Phase-contrast micrographs of uninduced 3T3-L1 cells on day 0 (A) and induced 3T3-L1 cells on day 4 (B) and day 10 (C). Oil Red-O staining was used to monitor oil accumulated on differentiated cells on plate (D). Triglyceride accumulation during differentiation of 3T3-L1 cells into adipocytes was measured (E).

monitored by microscopic observation, Oil Red-O staining and cell triglyceride assay. The un-induced 3T3-L1 cells have fibroblastic morphology (Figure 1A). After being treated with the adipogenic cocktail for 48 h, then transferred to fresh medium containing insulin for 8 days, most of 3T3-L1 preadipocytes were differentiated into adipocytes. By day 4, the cells became polygonal in shape and started to accumulate oil droplets (Figure 1B) that finally occupied most of the cytoplasm by day 10

(Figure 1C). The accumulated red oil droplets in the differentiated cells, incubated on Petri dishes were clearly visible after Oil Red-O staining (Figure 1D). The cytoplasmic triglycerides were released in the lysing buffer by sonication. The triglyceride concentration in the supernatant increased by 4.2-fold at day 10 (Figure 1E). These data were the confirmation of the 3T3-L1 preadipocyte being differentiated into adipocyte and was ready for hypoglycemic activity assays.

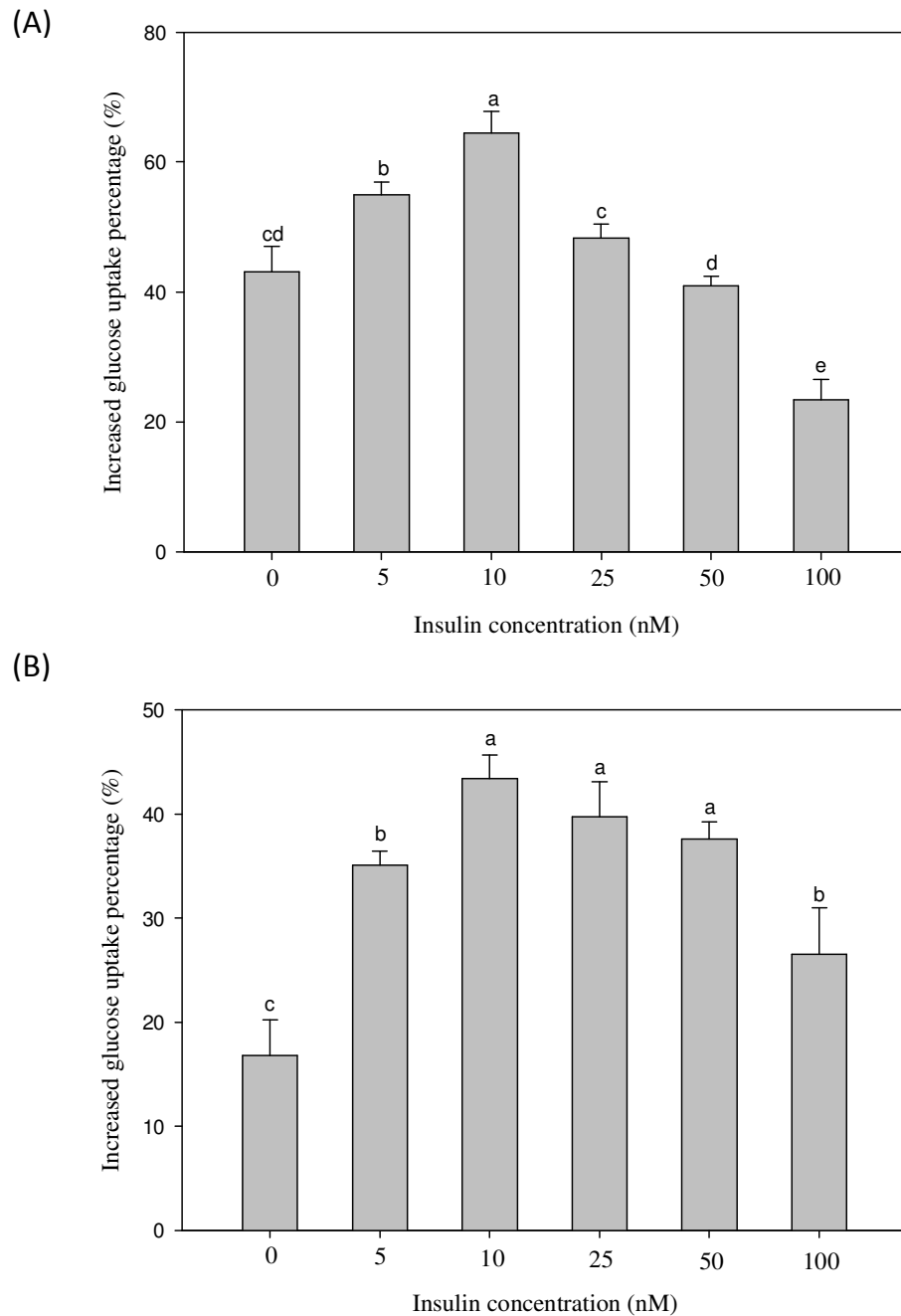


Figure 2. Effect of insulin concentration on the hypoglycemic activity of HGYE in SD model (A) and 3T3-L1 model (B). The hypoglycemic activity was expressed as the increased glucose uptake in percentage that was calculated as follow. Increased glucose uptake percentage (%) = $\{(\text{glucose uptake of cells with HGYE} - \text{glucose uptake of control}) / \text{glucose uptake of control}\} \times 100\%$. The bars represent the mean \pm S.D. (n=3). Different superscripts (a, b) on columns are significantly different ($p < 0.05$) according to Duncan's multiple range test.

Yeast screening by both models

The *in vitro* hypoglycemic activities were expressed as increased glucose uptake percentage in this study. 58

yeast strains isolated from various sources (grape, vineyard, and soils of winery) were obtained. The hypoglycemic activities were measured using both the SD model and 3T3-L1 model (Table 1). The hypoglycemic

Table 1. Hypoglycemic activity of yeast strains from various sources.

Strains number	Source	Hypoglycemic activity	
		SD	3T3-L1
1	Grape	-137.0	-79.1
2	Grape	-48.0	- 70.5
3	Grape	0.6	0.4
4	Grape	8.5	3.2
5	Grape	-17.0	- 1.4
6	Grape	30.5	31.4
7	Grape	16.0	13.3
8	Grape	21.0	- 17.1
9	Grape	-66.3	- 48.1
10	Grape	-11.3	- 24.3
11	Grape	-71.3	3.8
12	Vine Yard	-127.5	- 27.6
13	Vine Yard	10.9	32.9
14	Vine Yard	7.4	5.2
15	Vine Yard	-63.8	- 40.0
16	Vine Yard	5.6	18.6
17	Vine Yard	-41.3	23.8
18	Vine Yard	-70.0	- 11.9
19	Vine Yard	13.8	43.8
20	Vine Yard	-95.0	- 22.9
21	Vine Yard	-37.5	- 11.0
22	Vine Yard	-47.5	- 21.0
23	Vine Yard	-80.0	- 30.0
24	Vine Yard	-3.8	- 24.3
25	Vine Yard	-255.9	- 106.2
26	Vine Yard	9.9	- 2.4
27	Vine Yard	11.2	- 4.3
28	Vine Yard	-10.0	2.5
29	Vine Yard	21.8	24.8
30	Vine Yard	18.6	- 5.2
31	Vine Yard	8.0	2.4
32	Vine Yard	16.0	- 37.1
33	Vine Yard	- 148.3	- 14.75
34	Vine Yard	- 135.0	26.7
35	Vine Yard	- 206.3	- 51.4
36	Winery	52.3	12.0
37	Winery	- 110.0	- 10.5
38	Winery	15.2	- 2.9
39	Winery	6.8	- 1.0
40	Winery	1.3	0.6
41	Winery	1.2	9.4
42	Winery	13.3	3.8
43	Winery	36.5	24.3
44	Winery	14.9	- 2.4
45	Winery	9.3	8.1
46	Winery	6.6	- 14.3
47	Winery	10.4	- 5.7
48	Winery	- 73.8	- 9.1
49	Winery	5.3	1.9

Table 1 cont.

50	Winery	36.3	28.1
51	Winery	5.0	- 3.8
52	Winery	- 61.0	1.9
53	Winery	47.7	13.0
54	Winery	54.5	46.0
55	Winery	4.5	25.2
56	Winery	- 42.5	- 22.4
57	Winery	- 28.8	2.3
58	Winery	1.3	14.3

activity detection rates of the 58 yeast isolates were 56.9% (33/58) and 48.3% (28/58) by SD model and 3T3-L1 model respectively. Twenty two (22) strains were found to have hypoglycemic activity by both models. The strain no.54 possessed the highest activity measured by both models. The hypoglycemic activity as analyzed by SD model and 3T3-L1 model was 54.5 and 46% respectively. This strain (isolated from soils of a winery in Nantou, Taiwan) was identified to be *Saccharomyces pastorianus* by the Bioresource Collection and Research Center (Hsinchu, Taiwan). Accordingly, this strain was designated as *S. pastorianus* no. 54 and was used for the following studies. The above screening results suggested that the SD and 3T3-L1 models could be cross-referenced with each other in screening for yeast with hypoglycemic activity.

Effects of insulin concentrations

Cell extract of *S. pastorianus* no. 54 was used to compare the effects of insulin and HGYE concentrations on the hypoglycemic activity in both the SD and 3T3-L1 models (Figure 2). The hypoglycemic activity of HGYE in both models increased with increasing concentration of insulin and reached to maximum at 10 nM insulin, and dropped afterwards. At 10 nM insulin, the hypoglycemic activity obtained from SD model and 3T3-L1 model, was 64.5 and 43.4% respectively. The extract activity significantly decreased in the SD model at insulin concentrations higher than 10 nM (Figure 2A), which might have been caused by the phenomenon of insulin resistance (DeFronzo et al., 1979; Boden et al., 2005). In the 3T3-L1 model, insulin resistance phenomenon was also observed (Figure 2B).

Effects of HGYE concentration

Results obtained from the two models showed different HGYE dose responses in terms of the relative hypoglycemic activity (Figure 3). In the SD model, the activity increased at elevated concentration of HGYE, a 67.4% of increased glucose uptake percentage was

observed at 1000 µg/ml of HGYE (Figure 3A). In the 3T3-L1 model (Figure 3B), there was a steep increase of activity to 38.9% at 250 µg/ml of HGYE and the activity remained constant at higher HGYE concentration. This suggested that the 3T3-L1 model is more sensitive at the vicinity of 250 µg/ml. Linear relationship ($R^2 = 0.8611$) between the SD model and 3T3-L1 model was observed (Figure 4).

Hypoglycemic activity measurement by 2-(1-¹⁴C)deoxy-D-glucose

The amount of glucose uptake by cells, which was used for quantifying the hypoglycemic activity of HGYE, was indirectly determined by measuring the decrease in glucose concentration in the medium. Therefore, 2-(1-¹⁴C) deoxy-D-glucose (2-DG) was replaced with glucose to monitor the actual glucose uptake by 3T3-L1 adipocytes. The effect of insulin concentration on the yeast activity, expressed as increased 2-DG uptake percentage, is shown in Figure 5A. The activity of the HGYE at 250 µg/ml increased with increasing concentration of insulin, being highest at 10 nM insulin. Yeast activity significantly decreased when insulin concentration was increased to 100 nM. As shown in Figure 5B, the hypoglycemic activity increased with the increasing HGYE concentration and reached to the highest at 250 µg/ml. The profiles of insulin effect and dose response of HGYE, obtained in 3T3-L1 model by indirect measurement, as shown in Figures 2B and 3B, were in accordance with those obtained by direct measurement of ingested 2-DG, as shown in Figure 5. Due to easier operation and reliable data, the 3T3-L1 model by indirect measurement of the change in glucose concentration in the medium is recommendable for analyzing, *in vitro*, the hypoglycemic activity of HGYE.

Comparing the SD and 3T3-L1 models

In a study by Saito et al. (2008), it was demonstrated that sakuranetin, a flavonone from rice, could induce PPAR expression and reduce GATA-3 expression, and

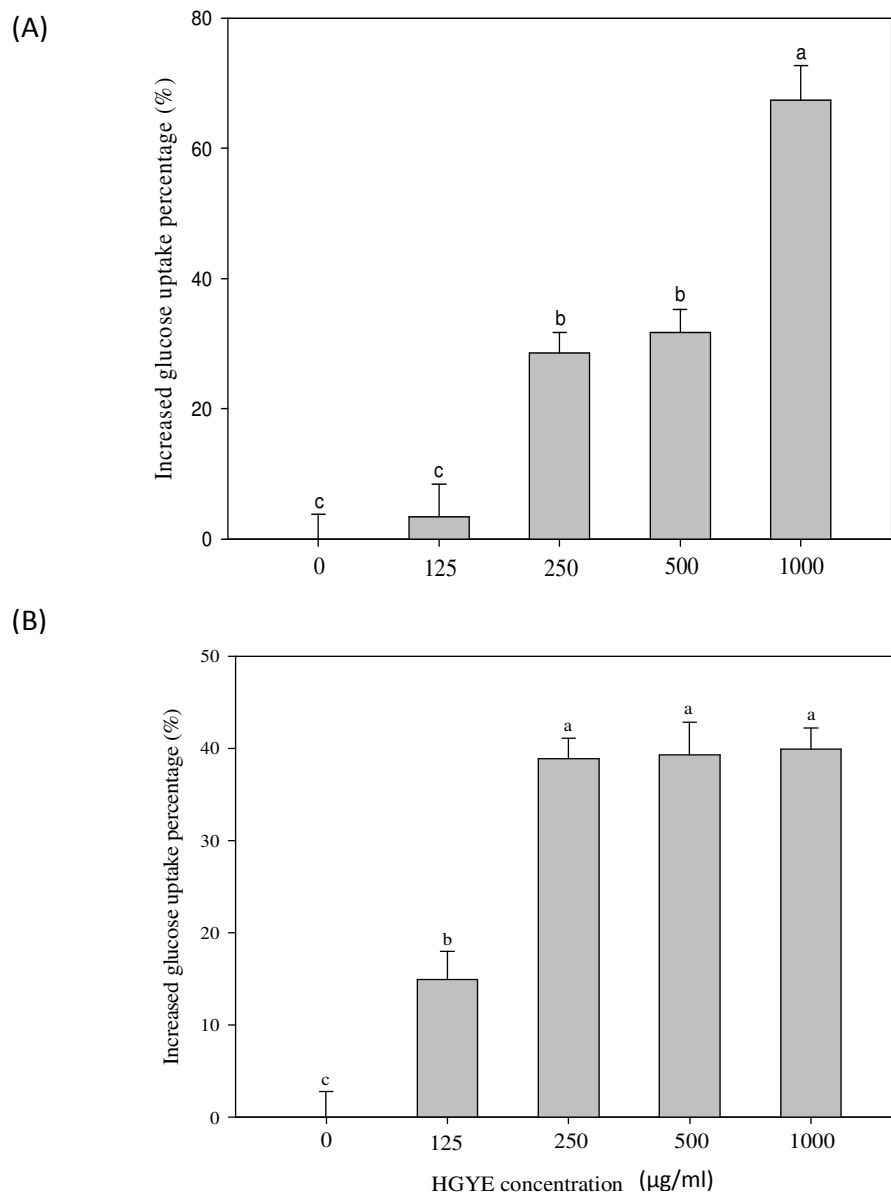


Figure 3. Effect of the concentration of HGYE on the hypoglycemic activity of HGYE in SD model (A) and 3T3-L1 model (B). The hypoglycemic activity was expressed as the increased glucose uptake percentage that was calculated as follow. Increased glucose uptake percentage (%) = $\{(\text{glucose uptake of cells with HGYE} - \text{glucose uptake of control}) / \text{glucose uptake of control}\} \times 100\%$. The bars represent the mean \pm S.D. (n=3). Different superscripts (a, b) on columns are significantly different ($p < 0.05$) according to Duncan's multiple range test.

consequently stimulate glucose uptake in 3T3-L1 adipocytes. Fulcher et al. (2008) have concluded the molecular mechanism of myosin IIA in mediating insulin-stimulated glucose uptake in 3T3-L1 adipocytes, via both the GLUT4 vesicle fusion at the plasma membrane and the activity of GLUT4 per se. In this study, we found that although the trends of dose response of hypoglycemic activity to HGYE were different between the SD model and 3T3-L1 model (Figure 3), a linear

relationship between these 2 models was observed (Figure 4).

Summary

The 3T3-L1 model used, in this study, for *in vitro* measurement of the hypoglycemic activity of yeast was found to be more cost effective and less time consuming.

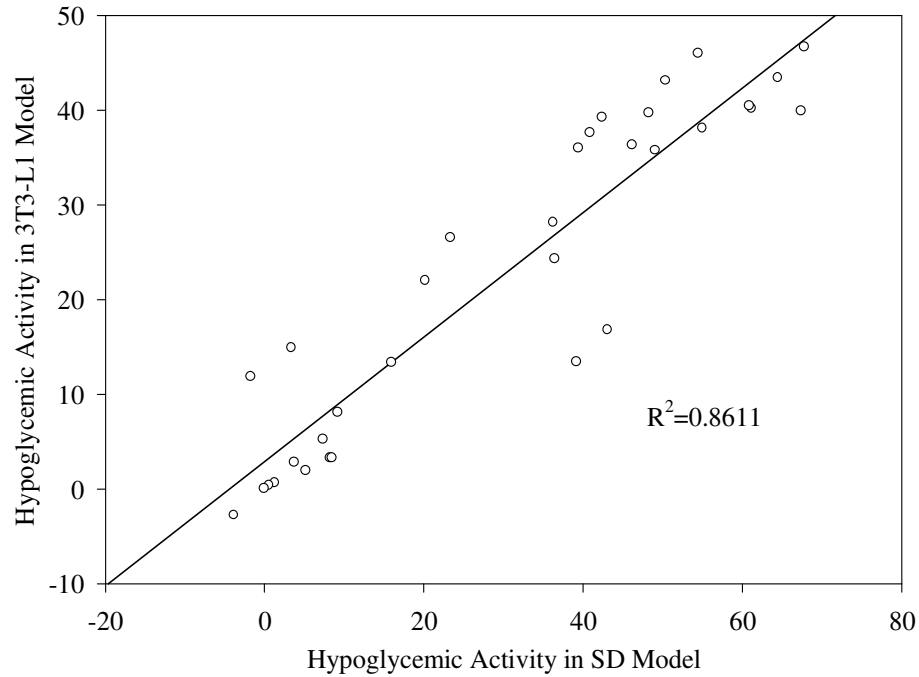


Figure 4. Correlation of the hypoglycemic activity of various extract concentrations of *S. pastorianus* no. 54 analyzed by SD model and 3T3-L1 model.

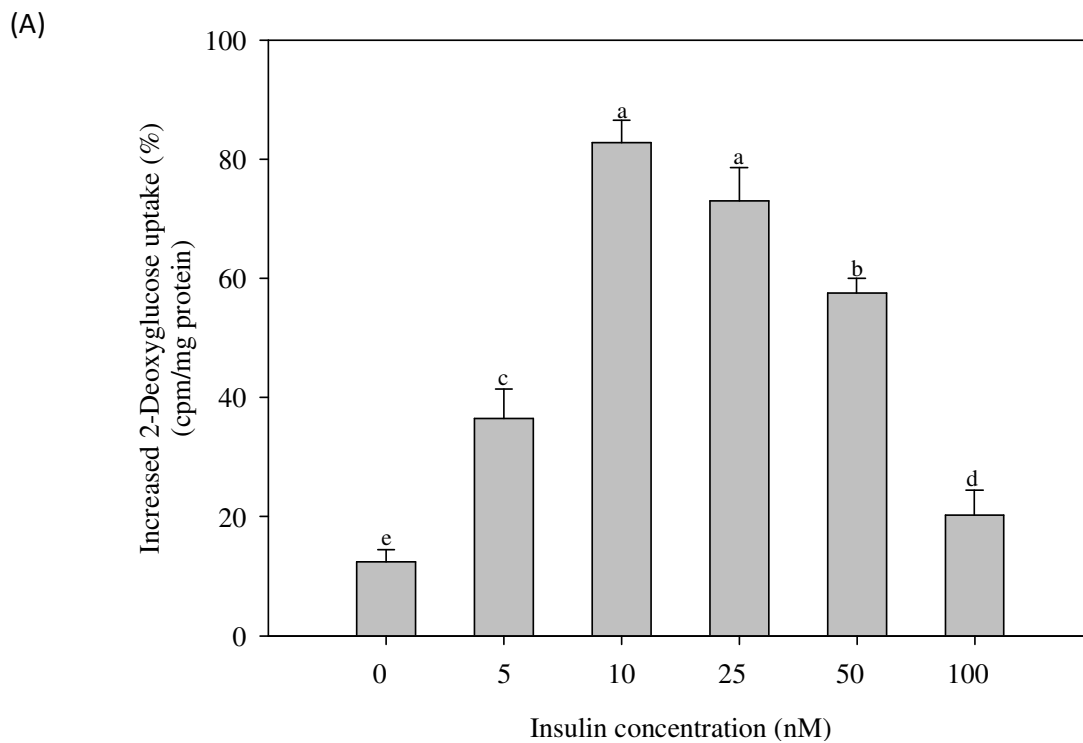


Figure 5. Effects of insulin concentration (A) and the concentration of HGYE on the increased 2-(^{14}C)deoxy-D-glucose (2-DG) uptake percentage in 3T3-L1 adipocyte. Increased 2-DG uptake (cpm/mg protein) (%) = $\{(\text{specific 2-DG uptake with HGYE} - \text{specific 2-DG uptake of control}) / (\text{specific 2-DG uptake of control})\} \times 100\%$. The bars represent the mean \pm S.D. (n=3). Different superscripts (a, b) on columns are significantly different ($p < 0.05$) according to Duncan's multiple range test.

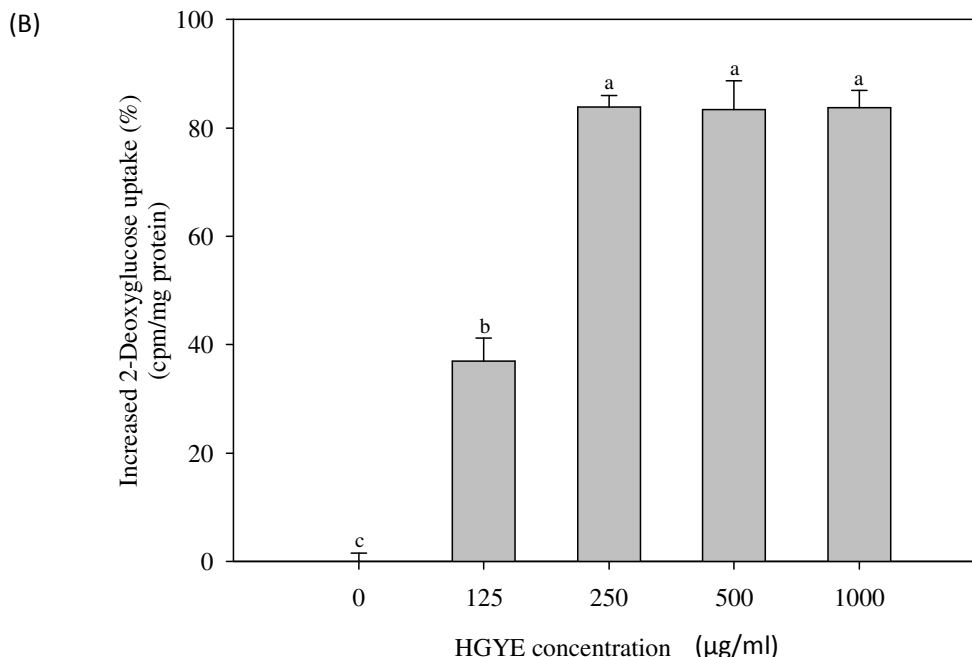


Figure 5. Continue.

The 3T3-L1 model showed higher activity at lower dosage of HGYE, and wider range of insulin concentration compared to the SD model. Validation of the 3T3-L1 model was verified by comparing the results, in the case of this study, of the two models which exhibited linear relationship between the two.

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